NANOTECHNOLOGY:
WHAT'S IN IT FOR BIOTECH?

At the nano level, physical, chemical, and biological properties of materials differ in fundamental and valuable ways. Bionanotechnology attempts to exploit these differences to create new biomedical research tools, diagnostic tests, and drug-delivery systems. The promise is great. So is the hype. How close are we? **BY JOHN MACK**

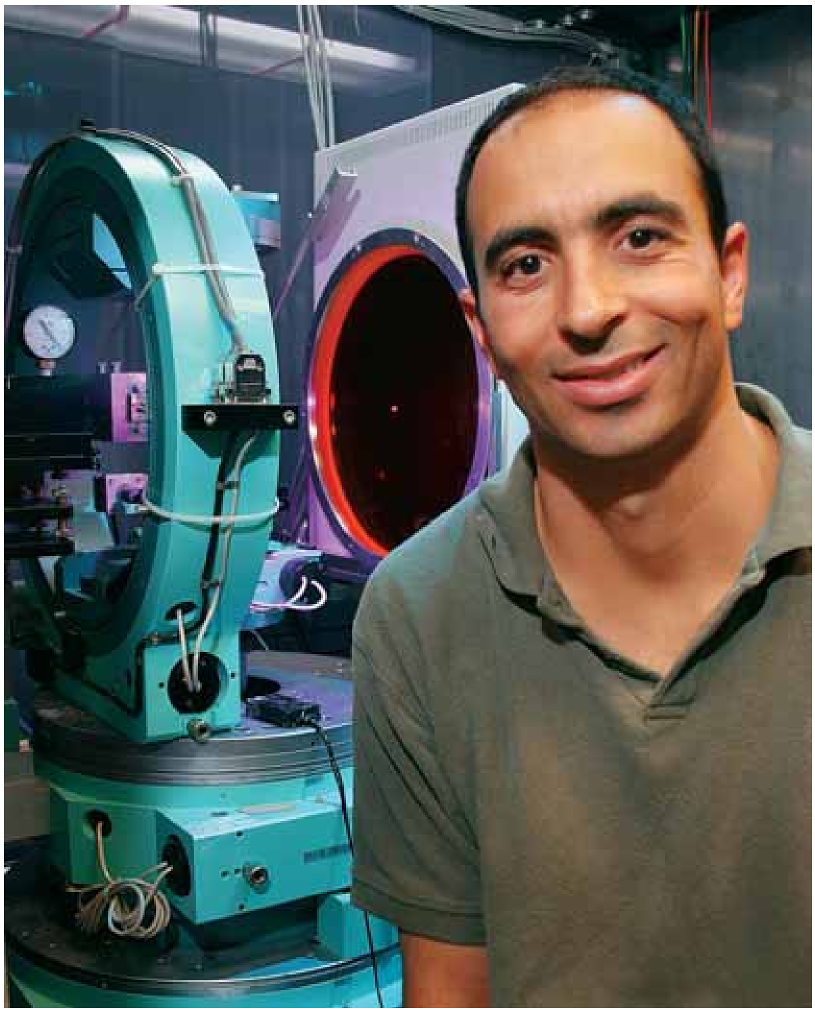
A "technology that does not yet exist" and "any sufficiently advanced technology is indistinguishable from magic" are phrases often quoted in describing nanotechnology. Recent advances in bionanotechnology, a biomedical branch of this burgeoning field, suggest the technology indeed exists.

Moreover, magic may be just what the pharma and biotech industries need now. Most drugs on the market have tremendous value and are priced right, according to many industry leaders. But, some cancer drugs and other biologic therapies developed by biotechs and their pharma partners are relatively costly and may add, on average, only a few months to the lives of patients using them. These and other drugs may be more effective if prescribed at earlier stages of disease or may have fewer side effects if delivered in lower effective doses.

Enter nanotechnology, which holds promise for reducing overall medical costs, improving outcomes, and adding value to drug therapy — mostly by increasing the efficiency of drug discovery, disease detection, and drug delivery.

JUST BUZZ — OR REALITY?

What *is* nanotechnology — that



PHOTOGRAPH BY ROD ROLLE

Biologists at UCSB are working with materials scientists to develop bionanotubes — tiny drug-delivery devices. Uri Raviv, PhD, a postdoctoral researcher, says that in the future, the tubes could deliver a drug or gene to a targeted location in the body.

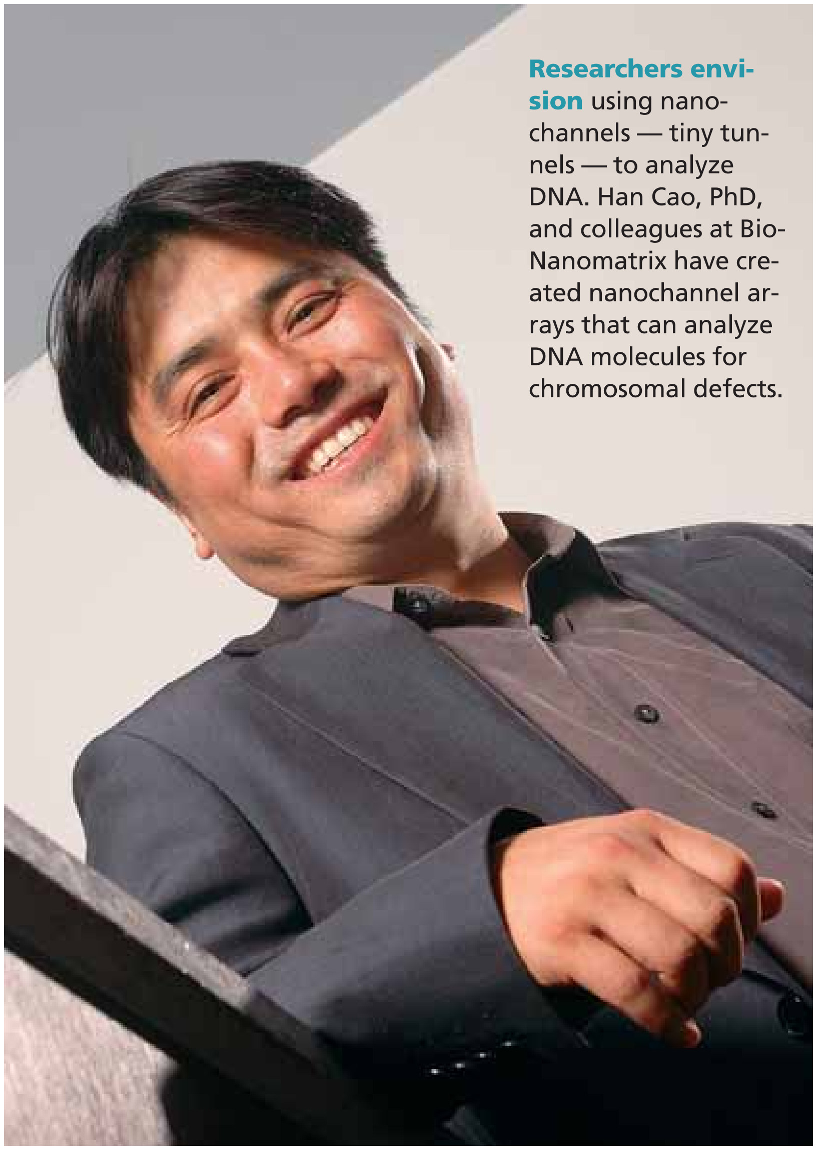
is, aside from a buzzword flying around the investment community these days? (Some researchers would contend that the physics and chemistry they've used for years are being glorified as nanotechnology merely to impress investors.)

According to the Center for Responsible Nanotechnology, a not-for-profit think tank concerned with the societal and environmental implications of nanotechnology, "The word *nanotechnology* has not yet acquired a common meaning. Widely disparate definitions can be found in dictionaries, organizational glossaries, and published documents.... Use of the term in science fiction, credible and fantastic, along with the sometimes questionable adoption of the word by research facilities and companies seeking funding or investment, contribute to this semantic dilution."

Simply stated, nanotechnology involves the interaction of cellular and molecular components and engineered materials. *Bionanotechnology*, then, typically applies this to atoms, molecules, and molecular fragments — together, the most elemental level of biology.

Whatever definition applies, think small. Very small. Objects in the nanoworld have dimensions that are between 1 and 100 nanometers, which is at least 1,000 times smaller than objects in the microworld. One micrometer (or micron), which is 1 millionth of a meter, equals 1,000 nanometers.

To appreciate how small the nanoworld is, consider the scale of a few materials: The width of a human hair is about 80 to 100 microns; red blood cells are approximately 2.5 microns in diameter; the width of a DNA molecule is 2 nm.



Researchers envision using nanochannels — tiny tunnels — to analyze DNA. Han Cao, PhD, and colleagues at BioNanomatrix have created nanochannel arrays that can analyze DNA molecules for chromosomal defects.

PHOTOGRAPH BY DAVID FONDA

What is important to remember is this: At the nano level, physical, chemical, and biological properties of materials differ in fundamental and valuable ways than we are used to. Bionanotechnology attempts to exploit these differences to create new biomedical research tools, diagnostic tests, and drug-delivery systems.

NANOTECH APPLICATIONS

Today's biomedical research technology rests firmly in the microworld and has revolutionized diagnostic tests and drug discovery. Yet this technology still is not sensitive enough for researchers like Han Cao, PhD, a molecular biologist interested in embryonic stem cell research. Cao is the founder and chief scientific officer at BioNanomatrix, a maker of nanodevices and systems for cancer diagnostics, complex disease therapeutics, and personalized medicine.

Cao laments that the technology is "lagging the needs of biomedical researchers. To do one experiment using the most sensitive technology available, it takes two months to accumulate enough sample because the cell is so rare. And if I drop the tube? Two months down the drain!" Far fewer cells would be needed if he could use nanotechnology in his experiments.

Where there is a need to analyze a small sample size, especially down to the level of a single cell or a molecule, an opportunity exists for using nanotechnology. Cao, for example, wants a technology that he could use to discern a few precancerous cells out of a heterogeneous cell population that is commonly found in biopsy samples.

Nanotechnology could help with early detection of some cancers, which depends on the detection of very small quantities of protein markers in the blood or on cell surfaces. This is the case with ovarian cancer, for which Inhibin A, a hormone, is possibly a marker. Current technology is not sensitive enough to detect this protein until the disease has progressed to an advanced stage, by which time the 5-year survival rate is less than 30 percent.

Nanoprobe technology. An example of a nanotechnology early-warning assay system is the biobarcode technology that is being developed by Nanosphere, a nanotechnology-based life sciences company in Northbrook, Ill. This technology, not yet commercially available, has potential for the diagnosis of Alzheimer's disease, prostate cancer, and other diseases.

The biobarcode assay is about 100,000 to 1 million times more sensitive than other available tests in the detection of ADDL, a protein that accumulates in the brains of Alzheimer's sufferers. The technology employs a gold nanoparticle probe with antibodies to the target protein attached to its polymer surface. It also carries a large number of unique, covalently attached oligonucleotides and complementary oligonucleotides. When the probe attaches to the target protein, the complementary oligonucleotides, which are the biobarcodes that serve as markers for the target protein, can be washed away. Because the nanoparticle probe carries many oligonucleotides per bound protein, there is substantial amplification, relative to protein.

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The challenge in applying this technology is to identify potential markers for a variety of diseases such as cancer, neurodegenerative diseases, infectious diseases, and cardiac and pulmonary diseases.

Lab on a chip. The so-called lab-on-a-chip technology currently being used in drug research and diagnostics is another example of a practical application of nanotechnology in medicine.

"Using lab-on-a-chip technology, massive numbers of samples can be run in parallel simultaneously," says Daniel Hayes, PhD, director of operations at NanoHorizons, which focuses on nanoscale applications in the biotech industry. "This allows more samples to be analyzed at lower cost with less potential for human error."

Roche Diagnostics, for example, developed the AmpliChip CYP450, the first FDA-approved microarray technology based test for comprehensive analyses of cytochrome P450 2D6 and 2C19, two genes that can influence drug efficacy and adverse drug reactions. The chip — no bigger than a thumbnail — contains more than 15,000 different oligonucleotide probes that can detect genetic variations in the cytochrome genes and enable highly accurate prediction of a patient phenotype (poor, intermediate, extensive, or ultrarapid metabolizer).

Test results will allow physicians to consider unique genetic information from patients before prescribing medications. The lowest effective dose then can be prescribed, thus reducing potentially harmful side effects that may show up at higher doses. Heino von Prondzynski, CEO of Roche Diagnostics, commented that "With this test it will, in certain cases, also be possible to prevent the selection of a less suitable or even harmful therapy."

Nanochannels. Researchers envision other forms of lab-on-a-chip technology. Cao, for example, anticipates using nanochannels to analyze DNA. Nanochannels resemble the long, narrow shape of DNA molecules. Cao and his colleagues at BioNanomatrix have been able to fabricate nanochannel arrays comprising hundreds of thousands of enclosed nanochannels with dimensions of 10 nm on a 100 mm wafer. Cao says it is possible to stream hundreds of thousands of DNA molecules through a nanochannel array in perfectly straight lines like a Eurostar train in the Channel Tunnel ("chunnel") between England and France. Except that the advantage, he adds, "would be the ability to distinguish potentially very densely packed biomarkers from one another along the DNA molecules — something that is difficult to do in solution where the molecules coil randomly.

"The key is to be able to do it in massive parallel 'chunnels' for high-throughput analysis and statistic significance. It's not just a single-file nanoscale analysis, as with a FACS [Fluorescent Activated Cell Sorter] machine."

Imagine a detector at a certain point along the nanochannel array. When the DNA trains pass through the "nano-chunnels," the detector will be able to isolate and examine each chromosomal defect, highlighted by markers such as fluorescent dyes attached to the gene.

Quantum dots. The use of fluorescent dyes to tag cells and cellular components goes back about 100 years. Dyes, however, are not as precise as biomedical researchers would like and, can be illuminated only for very short periods. Quantum dots — nanometer-sized semiconductor crystals that act like light emitting diodes — promise to revolutionize bioimaging.

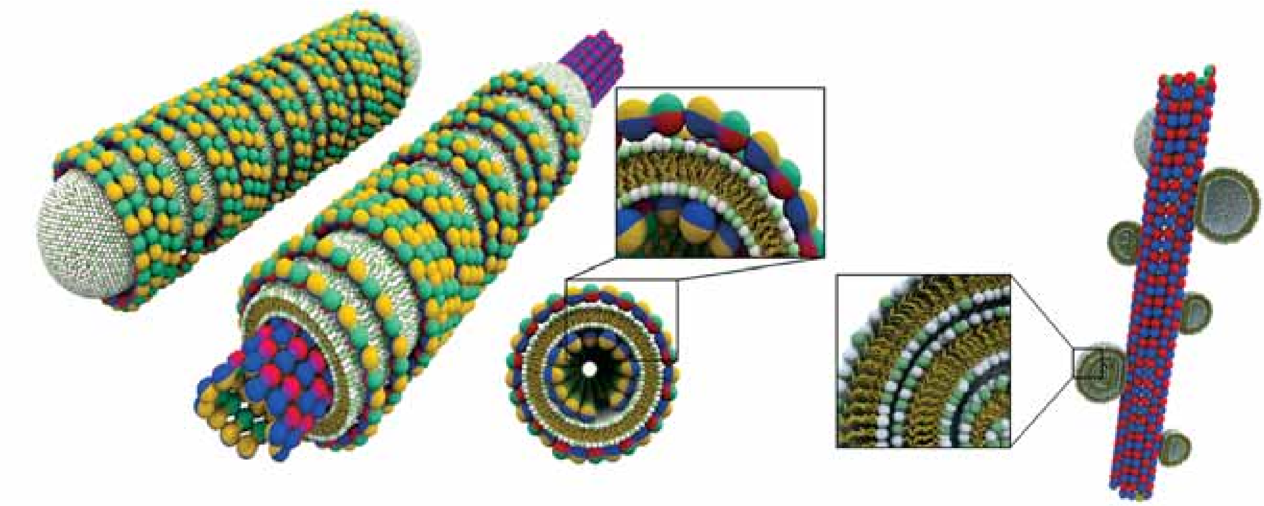
Quantum dots are used to light up specific biological events brightly and to allow rapid, parallel, and ultrasensitive detection of biomolecular interactions. A variety of targeting molecules, including monoclonal antibodies, can be attached to the polymer coating of quantum dots allowing them to attach to specific biomarkers.

According to the report "The Impact of Nanotechnology in Drug Delivery: Global Developments, Market Analysis and Future Prospects," by NanoMarkets, a market research firm, some of the world's largest pharmaceutical and biotech companies — GlaxoSmithKline, Pfizer, AstraZeneca, and Genentech, to name a few — are applying quantum dots in high-content drug screening.

Scientists at Genentech, for example, working in collaboration with Quantum Dot Corp., a leader in quantum dot biotechnology applications and products, succeeded in labeling the breast cancer marker HER2 with semiconductor quantum dots.

Because of their small size, quantum dots can function as cell- and even molecule-specific markers that will not interfere with the normal workings of a cell. The availability of quantum dots of different colors provides a powerful tool for following the actions of multiple cells and molecules simultaneously.

Drug delivery. Nanotechnologies such as lab-on-a-chip and



"Smart" bionanotubes are lipid-protein nanotubes made of microtubule protein (made of tubulin protein subunits, shown as red-blue-yellow-green objects) that are coated by a lipid bilayer (shown with yellow tails and green and white spherical heads), which in turn are coated by tubulin protein rings or spirals. An open-ended tube is shown in the center and a closed-end tube with a lipid cap is shown on the left. A top view of the nanotubes and a magnified region are shown on the right.

SOURCE: UNIVERSITY OF CALIFORNIA–SANTA BARBARA quantum dots are already well on the way to revolutionizing the drug-discovery process. Yet, the real promise of nanotechnology lies in its ability to target and facilitate drug delivery.

Technology being developed today for drug research and diagnostic tests also may be used in drug delivery. An implantable, silicon-based nanochannel drug delivery system, for example, may provide a way of delivering toxic drugs like interferon-alpha for the dosing of individual metastatic melanoma lesions without exposing the entire body to the powerful drug.

Researchers at Ohio State University recently constructed and tested a device composed of labyrinthine nanochannels etched in a crystalline silicon substrate. "The channel design," say the researchers, "leads to so-called zero-order drug release, that is, drug molecules exit the device at a constant rate that does not change as the amount of drug in the device drops over time."

Some researchers think that nanochannel drug delivery systems can be designed to release more or less drug, depending on in vivo conditions (e.g., releasing insulin in response to blood glucose levels) — someday. "Having a nano system that responds to the *in vivo* environment by releasing more or less drug is a very challenging technical problem," says Hayes. "I don't see it happening in the next few years."

REFORMULATED DRUGS

The simplest nanoenabled drug-delivery system involves the reformulation of drugs in nanoparticulate form. Wyeth, Merck, and Abbott all have developed FDA-approved nanoparticulate drugs using NanoCrystal technology developed by Elan Drug Delivery, a neuroscience-based biotechnology company located in Ireland.

For poor water-soluble compounds, such as Wyeth's sirolimus (Rapamune), NanoCrystal technology can enable formulation and improve compound activity and final product characteristics. By reducing the particle size of the drug to less than 200 nm, the total surface area of the dose is enlarged, overcoming the drug's relative insolubility. Previously available only as an oral solution in bottles or sachets, the new nanoreformulated sirolimus tablet was approved by the U.S. Food and Drug Administration in 2000.

DELIVERY APPLICATIONS

Materials scientists working with biologists at Univ. of California–Santa Barbara have developed "smart" bionanotubes that could be developed for drug- or gene-delivery applications (see page 35).

So-called smart bionanotubes are lipid-protein tubes with an inner space, for these experiments, measuring about 16 nm in diameter. The whole capsule is about 40 nanometers in diameter. Uri Raviv, PhD, a postdoctoral researcher involved in this research and a fellow of the International Human Frontier Science Program Organization, said the nanotubes are smart because "in the future, they could be designed to encapsulate and then open up to deliver a drug or gene in a particular location in the body."

By controlling the relative amount of lipid and protein, it is possible to switch between two states of nanotubes with either open ends or closed ends with lipid caps, a process that forms the basis for controlled chemical and drug encapsulation and release.

Raviv explains that the chemotherapy drug paclitaxel (Taxol) binds to microtubules and, therefore, could become one of the first drugs to be delivered by smart bionanotubes — in a manner specific to cancer cells — thereby reducing the side effects of chemotherapy treatments. "In principle," he says, "any drug that can get into the nanotubes without destroying them could be delivered to targeted cells."

Reformulating drugs like sirolimus and paclitaxel into better performing nanoparticulate form not only benefits patients, but can lead to patent extensions, which could be the ultimate payoff for the pharma industry.

SO, NOW WHAT?

"There is a lot of industry speculation and skepticism about where nanotechnology is going and how it will affect the pharmaceutical industry," says Mike Moradi, author of the NanoMarkets report cited previously. "2005 will be the first in which corporations will spend more money on nanotechnology research than will government or university labs," Moradi said.

New materials and new formulations will increase dosing efficacy, increase targeting ability, and eventually make the drug and biotech industry more profitable in the long term, though much research and development still has to be done to get it there. For now, it's all a bit magical. **BH**

John Mack, MS, MPhil, is a freelance writer/editor covering life science and technology issues.